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Limb Regeneration in the Eye Sockets of Crabs

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Abstract. The eyestalks of crabs were removed and various tissues of the limbs were autotransplanted into the empty eye sockets to study the capacity of the limb tissue to regenerate in a heterotopic site. Autotransplantation of walking leg tissues into the eye sockets was able to regenerate complete walking legs in the new site. Autotransplantation of tissues of claw digit (dactyl and pollex) or more proximal claw segments (ischium and merus/carpus joint) could regenerate complete claws in the eye sockets. If the autotransplant of claw tissue was contralateral, claws could regenerate with host-site handedness. Sham operations or autotransplantation of frozen claw tissue did not induce regeneration in the eye sockets. These results demonstrate that complete crab claws can regenerate from the eye sockets by autotransplantation of live limb tissue and that the regeneration is not due to the traumatic effect of transplantation.

The structure of the limbs regenerated in the eye sockets was determined by the source of the transplanted tissue. Complete claws resulted from autotransplantation of the tissues of the most distal claw segments (claw digits), and the most distal claw segments regenerated first, followed by the proximal claw segments in subsequent molts. Thus tissue from distal portions of crab claw can regenerate proximal portions of the claw in the eye sockets. Such a mode of regeneration is not consistent with the distalization rule of the polar coordinate model, which proposes that distal portions of the limb cannot regenerate proximal portions and that the direction of limb regeneration is always from proximal to distal.

Introduction

Our previous study (Kao and Chang, 1996) showed that autotransplantation of claw tissues into the autotomized stumps of crab walking legs can induce the stumps to regenerate claws or chimeras of claw and walking leg. The percentage of the claw or clawlike structures regenerated from the walking leg stumps was higher when the autotransplant consisted of a combination of claw tissues than when only a single type of tissue was used. With contralateral autotransplantation of claw tissues into the autotomized stumps of the walking leg, the stumps can regenerate claws with host-site handedness.

To further study whether claw tissues—singly or in combination—can regenerate complete claws in a heterotopic site that lacks a regenerating limb field, we autotransplanted claw tissues into the carapace at sites from which the eyestalks had been removed (these sites are hereafter called eye sockets). Crabs can regenerate their autotomized limbs but not their eyestalks (reviewed by Hopkins, 1988). There are no reports of crabs found in nature having limbs in the place of the eyestalks. The eye sockets thus provided an *in vivo* environment that was isolated from the tissue of the autotomized limb stumps.

In this study, we autotransplanted either single claw tissue or different combinations of claw tissues of the distal or more proximal claw segments into the eye sockets to determine whether there was any differential regenerative capacity. It has been proposed that the distal portions of the limbs cannot regenerate the proximal portions of the limbs (distalization rule of the polar coordinate model; Bryant *et al.,* 1981) and that different sizes or combinations of tissues can determine the regenerative capacity of hydra (Shimizu *et al.,* 1993) or the imaginal discs of *Drosophila* (Kauffman and Ling, 1981). In addition, we contralaterally autotransplanted limb tissues into the eye sockets to study whether the handedness of the regenerated limbs would be changed by the host site.

Materials and Methods

Animals and dissection

Two species of crabs were used: *Cancer gracilis* (range of carapace width = 6.9–27.9 mm, mean ± SD = 12.08

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± 3.49 mm, $n = 211$) and *C. jordani* (range of carapace width = 8.2–17.2 mm, mean ± SD = 11.55 ± 2.67 mm, $n = 12$). Specimens were collected subtidally with a hand net in Bodega Harbor, California. The crabs were kept in individual compartments to prevent cannibalism (Conklin and Chang, 1993) and were fed brine shrimp, shrimp meat, or fish every other day. Crabs were acclimated in the laboratory for at least one molt prior to experimentation. Only postmolt animals (less than 3 days after molt) were used.

Animals were forced to autotomize one limb—either a claw or the fourth walking leg. (Crab limbs are described and illustrated in Kao and Chang [1996].) Autotomy was achieved by crushing the manus or carpus segment of the limb with a pair of forceps. For donor tissues, the dactyl, pollex, ischium, and merus/carpus joint of the autotomized claw were used. In other experiments, the dactyl, ischium, and merus/carpus joint of the autotomized walking leg were used. These segments were cut off with a pair of fine scissors and placed into a depression slide with a drop of crab saline (440 m*M* NaCl; 11.3 m*M* KCl; 13.3 m*M* $CaCl_2$; 26 m*M* $MgCl_2$; 23 m*M* Na_2SO_4; 10 m*M* HEPES, pH 7.4 with NaOH; 100 units/ml penicillin; 0.1 mg/ml streptomycin; Cooke *et al.*, 1989). The slides were placed under a dissecting microscope and fine forceps were used to carefully pull intact tissues (including epidermis and muscle) out of the exoskeleton of each segment.

Removal of eyestalks and autotransplantation of limb tissues

One eyestalk of each crab (*C. gracilis*, $n = 211$; *C. jordani*, $n = 12$) was extirpated as proximally as possible with a pair of forceps under a dissecting microscope. This procedure left an opening at the site of entry of the eyestalk into the carapace (eye socket). Immediately after eyestalk removal, limb tissues were autotransplanted or sham operations were performed. The animals were then returned to seawater.

Autotransplantation consisted of pushing limb tissue into the eye socket, using the blunt end of an insect pin, until the tissue could no longer be seen with a dissecting microscope. Transplants were of two types: ipsilateral, in which claw tissue was removed from one side of the crab body and placed into the eye socket on the same side (*e.g.*, left claw tissues autotransplanted to the left eye socket of the same animal); and contralateral, in which the claw tissues were placed in the eye socket on the opposite side of the crab (*e.g.*, left claw tissues autotransplanted into the right eye socket of the same animal).

To examine the regenerative ability of different claw segments, single claw digits (dactyl or pollex), both claw digits (dactyl and pollex), or proximal segments of the claw (ischium + merus/carpus joint) were autotransplanted into eye sockets. To see whether the host site could regulate the morphology of the regenerated claw, different claw tissues (dactyl or pollex) or combinations of claw tissues (dactyl + pollex, ischium + merus/carpus joint, or dactyl + pollex + ischium) were contralaterally autotransplanted into eye sockets. To see whether dead tissues had the ability to induce regeneration in the eye socket, frozen claw tissues were autotransplanted into eye sockets.

To determine whether walking legs can regenerate from eye sockets, tissues from the dactyl, ischium, and the merus/carpus joint of the fourth walking legs were contralaterally autotransplanted into eye sockets. Control sham operations were conducted by inserting an insect pin three times into an eye socket. The transplantations were considered failures if no limb or limblike structure regenerated from the eye sockets after the fourth postoperative molt.

Characteristics of axes and handedness in C. gracilis

For the convenience of description, three axes of claws are defined. For the proximodistal axis, the proximal segments are the segments closest to the crab body. The distal segments are the segments farthest away from the body. Thus, the coxa and ischium are proximal segments of the claw; the dactyl and pollex are the distal "digits" of a claw. For the anteroposterior axis, the dactyl is the anterior movable "digit" of a claw and the pollex is the posterior fixed "digit" of a claw. For the dorsoventral axis, the dorsal surface of the claw manus has four ridges (carinae, Fig. 1A) along the proximodistal direction. The ventral surface of the claw is relatively smooth and lacks carinae (Fig. 1B). The dorsal surface of the walking leg has small granulosa tubercles and darker purple coloration (Fig. 1C), and the ventral surface of the walking leg is relatively smooth and lighter in color (Fig. 1D). The handedness of a claw can be easily distinguished by bending it. When the claw dactyl is oriented above the claw pollex, the right claw bends to the left and the left claw bends to the right. Handedness of the fourth walking leg can be identified by combinations of bending and axis characteristics. When viewed dorsally, the fourth right walking leg bends in a clockwise direction and the fourth left walking leg bends counterclockwise.

Results

Autotransplantation of limb tissues into eye sockets of C. gracilis

After autotransplantation of crab limb (claw or walking leg) tissues into crab eye sockets, limbs or limblike structures regenerated from some eye sockets (22.1% of sur-

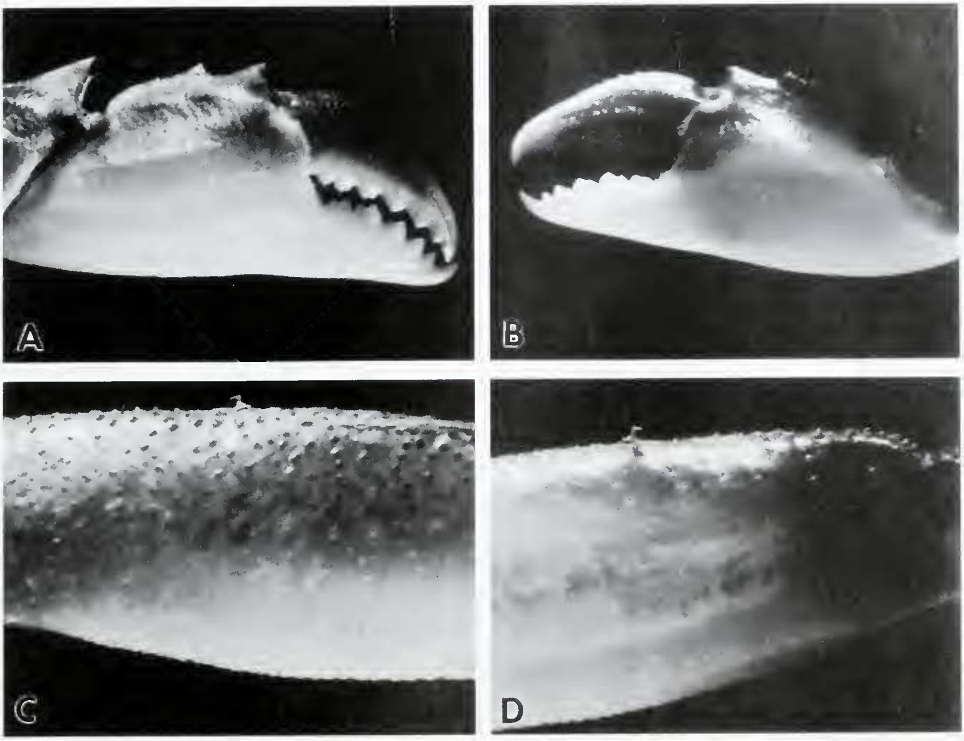

Figure 1. Characteristics of *Cancer gracilis* limbs. (A) Dorsal surface of claw manus has four ridges (carinae). (B) Ventral surface of claw manus does not have ridges. (C) Dorsal surface of the fourth walking leg has granulosa tubercles and darker pigmentation. (D) Ventral surface of the fourth walking leg does not have granulosa tubercles and has lighter pigmentation.

viving crabs that received live limb tissues, $n = 104$; Table I, Fig. 2A–I). According to our definitions, "regenerated" limbs contained at least two segments, and their handedness was recognizable; "limblike" regenerates consisted of one or more segments, but their handedness could not be recognized. Autotransplantation of claw tissues resulted in regeneration of claws or clawlike structures from eye sockets (20.6% of surviving crabs that received live claw tissues; Fig. 2A–H). Autotransplantation of fourth walking leg tissues resulted in regeneration of walking legs or leglike structures from the eye sockets (33.3% of the surviving crabs; Fig. 2I). Sham-operated crabs or autotransplantation of frozen claw tissues into eye sockets did not regenerate any limb or limblike structure from the sockets (Table I).

The number of crab molt cycles needed to regenerate these structures and the number of claw segments regenerated per molt were variable among crabs. Regeneration of claws with complete segments (Figs. 2A, B, 3A) usually took two to four postoperative molts. In most cases, crabs regenerated one or two distal segments after the second postoperative molt (Fig. 2A, B) and the rest of the proximal segments after the third or fourth postoperative molts. Some crabs, however, regenerated complete claws after the second postoperative molt (Fig. 3A). Both sham-operated and autotransplanted crabs regenerated normal limbs from their autotomized stumps after the first postoperative molt regardless of whether limbs (or limblike structures) were regenerating in their eye sockets. The duration of the first postoperative molt interval of the sham-operated (22.44 ± 1.08d, $n = 18$) and experimental (20.8 ± 0.56d, $n = 91$) animals was variable among individuals of similar sizes and not statistically different between the two groups ($P = 0.094$).

Table I

Summary of limb tissue autotransplantation in the eye socket of Cancer gracilis *(carapace width = 12.05 ± 3.40 mm)*

				* Type of regenerate	
Donor tissue	Host eye	Trials (No.)	Survival (No.)	Limblike	Limb
Claw					
dactyl	ipsi	15	6	0	0
dactyl	contra	17	8	1	0
pollex	ipsi	15	8	2	0
pollex	contra	10	3	1	0
dactyl + pollex	ipsi	19	13	2	2
dactyl + pollex	contra	23	15	1	1
frozen dactyl + pollex	ipsi	12	9	0	0
dactyl + pollex + ischium	contra	20	15	1	3
ischium + joint	ipsi	23	14	1	1
ischium + joint	contra	13	10	1	2
Walking leg					
dactyl + ischium + joint	contra	19	12	3	1
Sham operation	ipsi	25	18	0	0
Total		**211**	**131**	**13**	**10**

* Limblike: a structure that resembles a claw or walking leg and in which handedness cannot be recognized; limb: a claw or walking leg in which handedness can be recognized.

Both distal and proximal limb tissues can regenerate complete limbs

Autotransplantation of a combination of distal and proximal claw segments (dactyl + pollex + ischium) into eye sockets regenerated claws or clawlike structures in four cases (26.7% of surviving crabs, $n = 15$). Among them, three were claws (Fig. 2A, B) and one was a tapered projection. The claws had both claw digits and at least two segments. In addition, the digits had toothlike structures. The tapered projection had neither a joint nor any teeth. Its size increased after the crab molted, but the shape had not changed after four postoperative molts.

Autotransplantation of proximal segments of claws (ischium + merus/carpus joint) or distal parts of claws (dactyl + pollex) regenerated claws or clawlike structures (11 cases, 21.2% of surviving crabs, $n = 52$). Among them, six were claws (Fig. 2C) and five were clawlike structures (Fig. 2D–F). The clawlike structures included Y-shaped (Fig. 2D), pollexlike structures (Fig. 2E), and a structure similar to the manus and carpus segments of the claw (Fig. 2F). The Y-shaped limb (Fig. 2D) resulted from contralateral autotransplantation of dactyl and pollex tissues of the claw. The pollexlike structure (Fig. 2E) regenerated from lateral autotransplantation of dactyl and pollex tissues of the claw. It had two rows of teeth but no pigmentation. The structure similar to the claw manus and carpus segments (Fig. 2F) resulted from autotransplantation of the ischium and merus/carpus joint of the claw.

Autotransplantation of single digits of claws (dactyl or pollex) regenerated tapered projections, characterized by the lack of a joint, in four cases (16% of surviving crabs, $n = 25$; Fig. 2G, H). These crabs failed to regenerate claws after four postoperative molts. Autotransplantation of fourth walking leg tissues (dactyl + ischium + merus/carpus joint) regenerated one complete walking leg (Fig. 2I) and three incomplete walking legs that contained only distal segments (dactyl or dactyl + manus) of the fourth walking leg.

Two modes of regeneration in eye sockets after autotransplantation of crab limb tissues

Two modes of regeneration were observed. One mode—regeneration of a complete claw following ecdysis—can occur in autotransplantation of both digits of the claw, the proximal segments of claws (ischium + merus/carpus joint), or a combination of distal and proximal segments of claws (dactyl + pollex + ischium). In this form of regeneration, a budlike structure emerged from the eye socket before ecdysis, but no limb or limblike structures were observed at that time. After ecdysis, a complete claw regenerated from the eye socket.

The second mode of regeneration occurred in autotransplantation of a combination of distal and proximal claw tissues (dactyl + pollex + ischium) or of both claw digits (dactyl + pollex), but not in autotransplantation of the proximal claw segments (ischium + merus/carpus joint). In this mode, distal segments (dactyl or dactyl + pollex; Fig. 2C) regenerated first, followed by the appearance of proximal segments after subsequent molts. For the claw shown in Figure 2B, for example, we observed a dactyl emerge from the eye socket after the second postoperative molt. Both the dactyl and pollex were present in the eye socket after the third postoperative molt; a complete claw regenerated from the eye socket after the fourth postoperative molt. To confirm that only distal claw segments initially regenerated in the eye sockets, some crabs were dissected immediately after the distal segments first emerged from the eye sockets, and crab exuviae were examined at successive postoperative molts. We found no claw segments hidden inside the carapace that could not be observed from the exterior under a dissecting microscope, and we confirmed that those initially regenerated claw segments contained only distal claw segments. Those crabs contained either a single claw digit (dactyl), both claw digits (dactyl and pollex), or claw digits and a partial manus segment (Fig. 2C).

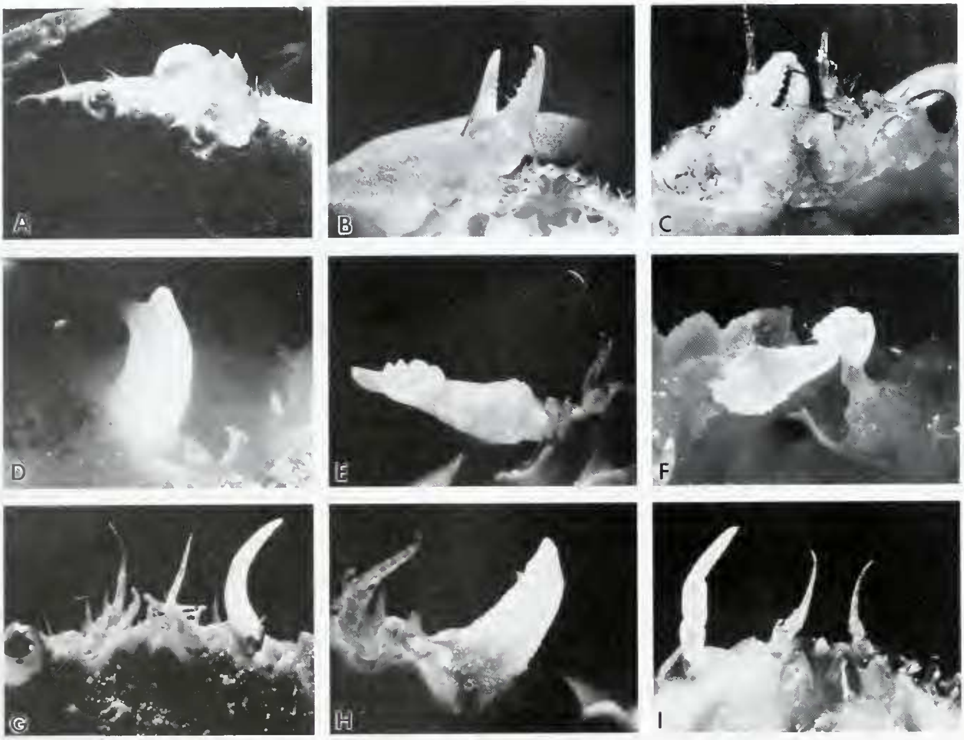

Figure 2. Limb or limblike structures regenerated from *Cancer gracilis* eye sockets. (A) Complete claw regenerated from the eye socket after the fourth postoperative molt (M4). (B) Complete claw with a mixture of donor and host-site handedness after M4. (C) Claw with dactyl, pollex, and partial manus segments after the second postoperative molt (M2). (D) Y-shaped limb after M2. (E) Pollexlike structure after the third postoperative molt. (F) Clawlike structure with manus and carpus segments after M2. (G, H) Distally tapered regenerates after M4. (I) Complete walking leg after M2.

Handedness of regenerated limbs in C. gracilis

Contralateral autotransplantation of claw tissues into eye sockets regenerated six claws with recognizable handedness. Among them, two claws had host-site handedness (Fig. 2A), three claws had donor-tissue handedness, and one claw had a mixture of host and donor handedness (Fig. 2B). The two claws with host-site handedness resulted from contralateral autotransplantation of a combination of distal and proximal claw tissues (dactyl + pollex + ischium; Fig. 2A), or proximal claw tissues (ischium + menus/carpus joint). The claw with a mixture of host and donor handedness resulted from autotransplantation of a combination of distal and proximal tissues (dactyl + pollex + ischium; Fig. 2B). It had donor handedness at its distal parts and host handedness at the proximal parts of the claw. The claw digits regenerated first, and the proximal segments regenerated during subsequent molts. Ipsilateral autotransplantation of claw tissues regenerated three claws with recognizable handedness. These three claws retained the donor (same as the host-site) handedness (Fig. 2C). Contralateral autotransplantation of the fourth walking leg tissues into eye sockets regenerated one walking leg with recognizable, donor-tissue handedness (Fig. 2I).

Autotransplantation of claw tissues into eye sockets in C. jordani

Contralateral autotransplantation of *C. jordani* claw tissues into eye sockets regenerated one complete and one incomplete claw (Table II). The complete claw (Fig. 3A)

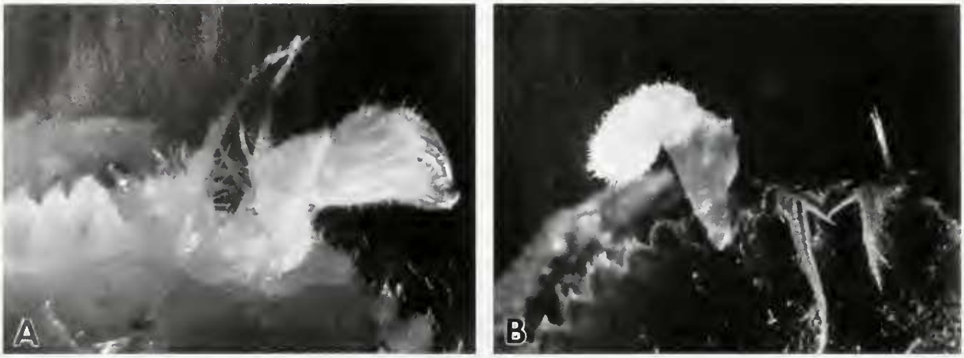

Figure 3. Claw regenerated from *Cancer jordani* eye sockets. (A) Complete claw regenerated from the eye socket after the second postoperative molt (M2). (B) Incomplete claw with all segments proximal to the claw digits regenerated after M2.

resulted from contralateral autotransplantation of both claw digits and had host-site handedness. Like an authentic claw, it contained five segments. The incomplete claw (Fig. 3B) resulted from contralateral autotransplantation of the claw pollex and had donor-tissue handedness. It had a fork-shaped structure at the distal tip and four normal proximal segments. The fork-shaped structure was regenerated first, and the proximal segments were regenerated in subsequent molts. All sham-operated animals failed to regenerate limbs in eye sockets.

Discussion

Kim and Stocum (1986) autografted anterior or posterior halves of axolotl forearms to eye sockets and amputated them distally 7 days later. The transplants could not regenerate proximal structures of the limbs. They either regressed until a small fragment of the radius remained in the orbit or regenerated no more than two digits distally. Pecorino *et al.* (1996) transplanted the hindlimb distal blastema to the forelimb proximal stumps of newt limbs. The proximal part of the regenerate was mostly generated by the stump, and the transplanted cells made only a minor contribution. These results, together with older studies (Dent, 1954; Butler, 1955), indicate that amphibian limbs can regenerate distally but not proximally, and they are consistent with the distalization rule of the polar coordinate model (Bryant *et al.,* 1981).

Table II

Summary of limb tissue autotransplantation in the eye socket of Cancer jordani *(carapace width = 10.79 ± 4.05 mm)*

Donor tissue	Host eye	Trials (No.)	Survival (No.)	Claw
pollex	contra	1	1	1
dactyl + pollex	contra	8	4	1
Sham operation	contra	3	2	0

When we autotransplanted claw tissues into the eye sockets, the transplanted tissues were completely inside the crab body. This is in contrast to the transplantation methods used in amphibians, in which only the most proximal portions of the limbs or regeneration blastemas were inserted into the body. Our results showed that complete claws can regenerate from the eye sockets by autotransplantation of tissues of either distal (dactyl and pollex) or more proximal claw segments. Even autotransplantation of a single claw digit (pollex) resulted in regeneration of a claw with complete proximal segments (ischium, merus, carpus, and manus) but with incomplete distal segments (without a dactyl or pollex, Fig. 3B). In addition, we also observed that distal claw segments can regenerate initially, followed by regeneration of proximal segments after subsequent molts. These results demonstrate that complete claws can regenerate from crab eye sockets by autotransplantation of tissues of the distal claw segment and that claw regeneration in the eye sockets can proceed in a distal to proximal direction. Such a mode of regeneration is inconsistent with the results observed in amphibians and with the distalization rule of the polar coordinate model (Bryant *et al.*, 1981). This inconsistency may be due to differences in the grafting methods or species used.

Although antenna-like structures or antennules growing from eye sockets have been observed in both wild and experimental decapods (Bateson, 1894; Maynard, 1965; Nevin and Malecha, 1991), this phenomenon has not been reported for crabs. We also did not observe such structures in our experimental or sham-operated crabs. Some animals regenerated tapered projections (Fig. 2G, H) or structures different from the original grafted tissues in the eyes sockets (Fig. 2E, F). They were, however, neither an antennule nor an antenna, because both appendages had multiple segments. Autotransplantation of a single claw digit into eye sockets resulted in a low percentage of regenerates in the eye socket, and all the regenerates were tapered or distally incomplete. We believe that the claw tissues in the eye sockets underwent regression and that regeneration of a complete distal structure is dependent upon a complete proximal claw structure. This obeys the complete circle rule of the polar coordinate model (French *et al.,* 1976; Bryant *et al.,* 1981).

Autotransplantation of a claw pollex into an eye socket regenerated a claw with incomplete distal segments but complete proximal segments. A Y-shaped structure initially emerged from the eye socket after the first postoperative molt, and a claw with complete proximal segments but incomplete distal segments regenerated from the eye socket after the second postoperative molt. This result suggests that claw proximalization, unlike distalization, need not have a complete structure at the distal tip. Limb tissues grafted into eye sockets did not regenerate eye structures. Instead, only limb structures regenerated from eye sockets. Our results showed that limb tissues are not pluripotent; their fates have been determined to regenerate limbs even in the eye sockets.

We observed that claw regeneration in the eye sockets can proceed from the distal to the proximal parts of the claws (Fig. 2C, J). This process can occur over four molt cycles. Examination of dissected animals and crab exuviae revealed that this mode of regeneration is produced by continuously generating blastemas at the proximal ends of the previous regenerates. The proximal ends of blastemas and future regenerated claws were not suspended in the crab body. Instead, they fused with tendons of eyestalks (Fig. 3A), remnants of eyestalks (Fig. 2F), or the carapace near the eye sockets (Fig. 2C). This explains why they were not shed with the old exoskeleton during molt. We observed that although the proximal segments of the regenerated claws were capable of movement, the dactyl was not. Thus they were not fully functional claws.

In our experiments, a total of 12 limbs (11 claws and 1 walking leg) with recognizable handedness regenerated from the crab eye sockets. Among them, 8 limbs (7 claws and 1 walking leg) retained the donor-tissue handedness regardless of whether the autotransplantation of claw tissues was ipsilateral or contralateral. Three regenerated claws changed from donor-tissue to host-site handedness, and one regenerated claw had a mixture of donor and host handedness following contralateral autotransplantation of claw tissues into eye sockets. The mechanism for change of handedness of crab claws is unknown, but our observations suggest that the axis of grafted tissues in the eye socket has been changed.

Handedness of limbs is determined by the dorsoventral, proximodistal, and anteroposterior axes. Left and right hands have the same dorsoventral and proximodistal axes but are opposite in the anteroposterior axis. One explanation for the change of claw handedness is that the grafted claw tissues in the eye sockets have been reorganized. It is known that limb regeneration does not take place by direct outgrowth but by the production of undifferentiated blastema cells. The blastema cells are derived from dedifferentiation of stump tissues (Adiyodi, 1972; Stocum, 1991; Tsonis *et al.,* 1995). Since we grafted a piece or several pieces of claw tissues into eye sockets, the dedifferentiation process might occur in all pieces of the transplants. Dedifferentiation of limb tissue might generate a new limb primordia in which the axes are undetermined. With a complete dedifferentiation of claw tissues in eye sockets, the handedness of claws might be determined by the host site. Without a complete dedifferentiation of claw tissues in eye sockets, the handedness of the regenerating claw might be inherited from the original donor tissues. A mixture of donor and host handedness may be due to the influence of both the donor tissues and the host site. Alternatively, it is possible that the handedness of the claws regenerating in the eye sockets might be determined by a random process unrelated to the handedness of the grafted tissues or host sites.

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Literature Cited

Adiyodi, R. G. 1972. Wound healing and regeneration in the crab, *Paratelphusa hydrodromous. Int. Rev. Cytol.* **32:** 257–289.

Bateson, W. 1894. *Materials for the Study of Variation Treated with Special Regard to Discontinuity in the Origin of Species.* Macmillan, London. 598 pp.

Bryant, S. V., V. French, and P. J. Bryant. 1981. Distal regeneration and symmetry. *Science* **212:** 993–1002.

Butler, E. G. 1955. Regeneration of the urodele forelimb after reversal of its proximo-distal axis. *J. Morphol.* **96:** 265–281.

Conklin, D. E., and E. S. Chang. 1993. Culture of juvenile lobster (*Homarus americanus*). Pp. 497–510 in *CRC Handbook of Mariculture,* 2nd ed, J. P. McVey, ed. CRC Press, Boca Raton, FL.

Cooke, I., R. Graf, S. Grau, B. Haylett, D. Meyers, and P. Ruben. 1989. Crustacean peptidergic neurons in culture show immediate outgrowth in simple medium. *Proc. Natl. Acad. Sci. USA* **80:** 402–406.

Dent, J. N. 1954. A study of regenerates emanating from limb transplants with reversed proximodistal polarity in the adult newt. *Anat. Rec.* **118:** 841–856.

French, V., P. J. Bryant, and S. V. Bryant. 1976. Pattern regulation in epimorphic field. *Science* **193:** 968–981.

Hopkins, P. 1988. Control of regeneration in crustaceans. Pp. 327–340 in *Endocrinology of Selected Invertebrate Types,* H. Laufer and R. G. H. Downer, eds. A. R. Liss, New York.

Kao, H.-w., and E. S. Chang. 1996. Homeotic transformation of limb by autotransplantation of crab claw tissue. *Biol. Bull.* **190:** 313–321.

Kauffman, S. A., and E. Ling. 1981. Regeneration by complementary wing disc fragments of *Drosophila melanogaster. Dev. Biol.* **82:** 238–257.

Kim, W.-S., and D. L. Stocum. 1986. Retinoic acid modifies positional memory in the anteroposterior axis of regenerating axolotl limbs. *Dev. Biol.* **114:** 170–179.

Maynard, D. M. 1965. The occurrence and functional characteristics of heteromorph antennules in an experimental population of spiny lobsters, *Panulirus argus. J. Exp. Biol.* **43:** 79–106.

Nevin, P. A., and S. R. Malecha. 1991. The occurrence of a heteromorph antennule in a cultured freshwater prawn, *Macrobrachium rosenbergii* (De Man) (Decapoda, Caridea). *Crustaceana* **60:** 105–107.

Pecorino, L. T., A. Entwistle, and J. P. Brockes. 1996. Activation of a single retinoic acid receptor isoform mediates proximodistal respecification. *Curr. Biol.* **6:** 563–569.

Shimizu, H., Y. Sawada, and T. Sugiyama. 1993. Minimum tissue size required for hydra regeneration. *Dev. Biol.* **155:** 287–296.

Stocum, D. L. 1991. Limb regeneration: a call to arms (and legs). *Cell* **67:** 5–8.

Tsonis, P. A., C. H. Washabaugh, and K. Del Rio-Tsonis. 1995. Transdifferentiation as a basis for amphibian limb regeneration. *Semin. Cell Biol.* **6:** 127–135.